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(54) House dust mite allergen control

(57) House dust mite allergens are controlled by coating a surface with a composition containing a material capable of binding small particles so as to immobilise allergens on the surface. Preferred binding materials are polysaccharides. Particularly suitable are water soluble polysaccharides such as hydroxypropylmethyl cellulose. The composition may also contain a wetting agent and a preservative.

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HOUSE DUST MITE ALLERGEN CONTROL

The invention relates to the control of house dust mite allergen and, more particularly, to the use of polysaccharides, especially aqueous solutions thereof, for the control of these allergens.

The house dust mite plays a dominant role in both the actual induction of asthma and the subsequent triggering of wheezing attacks. Asthma is primarily an allergic reaction of the lungs to various airborne particles of which the faeces of the house dust mite, whole or crushed, are by far the most important. The same allergen is responsible for allergic rhinitis.

The nature of the allergen is well understood. It is one of the protease enzymes produced by the mites and secreted into their mid-guts to enable them to digest the protein in their food. The enzyme survives passage through the gut and is found in the faecal pellets of the mite. It is a water soluble glyco-peptide, still capable of functioning as a protease and is a very powerful allergen.

The population levels of mites are normally controlled by the humidity in a building. Food needs to be moist enough to provide them with at least the bare minimum of essential water if they are to survive. Mites

feed on the microscopic fragments of human skin which are initially too dry for the mites to use as they fall off the human body, however if they lie in a moist atmosphere for a few days, they absorb water from the air, often helped by miniature mould growths growing on them. The resultant moist soft skin scale is more suitable as mite food.

Only the most structurally damp houses will be suitable for house dust mites to flourish everywhere. A typical centrally heated U.K. dwelling will support dust mites in upholstery which is usually full of skin fragments and the moisture given off by someone sitting on a chair will provide some of the water which the mites require. Allergen is puffed into the air when people sit on the upholstery.

Mites will thrive, however, in one place in the house regardless of the ambient humidity in the house and that is in beds and bedding.

Asthma is a contact allergy. Solid particles containing allergen have to make contact with the mucosa lining the bronchi of the lungs. If this does not happen then there cannot be any symptoms. The only way in which contact can be made is by breathing in the allergen. Severity of asthmatic symptoms depends in part on how sensitive the sufferer is to the allergen and partly on

the concentration of available allergen. The most important way in which allergen becomes airborne is the squeezing of soft furnishing. Sitting down on upholstered chairs, turning over a mattress, and putting weight on a pillow, will all puff allergen into the air.

Dust mite faecal pellets are smaller then most pollen grains and are also lighter as they are drier and are therefore easily airborne. Good hygiene practices are essential in the fight against dust mite and allergens.

Tannic acid has been used for a number of years as a method of combating allergen particles and was reported by Green WF, "Abolition of allergens by tannic acid" in the Lancet 1984 ii 160. Commercial products made from tannic acid are generally solutions in the form of a spray. It is claimed that these products denature the allergen proteins, making them insoluble whilst at the same time altering their surface area so that they no longer bond with the antibodies sent by the human immune system to destroy them. Thus the altered shape becomes unrecognisable by the immune system's memory, preventing the usual allergic reaction from taking place.

One disadvantage of the use of tannic acid is the fact that it can discolour a treated fabric over a period of time.

It is an object of the invention, therefore, to find

a range of compounds which, preferably, are able to control the dust mite allergen whilst remaining stable on the applied surfaces. Such a range of compounds would also preferably be of essentially natural origin, be non-toxic and water soluble so that treated fabric surfaces could be washed easily as part of good hygiene practice. It is a further object of the invention to provide an improved method of controlling dust mite allergen.

The invention provides a composition for controlling dust mite allergens, comprising a coating material able to bind small particles and which can be applied to a surface so as to substantially immobilise allergen particles on the surface. Such a composition prevents allergic reactions by minimising the exposure of asthma suffers to the allergen particles and does not rely on denaturing the allergen particles.

The coating material is preferably water soluble and may be applied as an aqueous solution by spraying onto the surfaces. Alternatively, any suitable textile coating technique may be used such as pad mangling. Spraying has the advantage, of being quick and simple and can be done in the home.

The coating material may be any one of a number of substances which are able to bind small particles, eg. polyvinylalcohols (PVA), however, a preferred coating

material is a polysaccharide. The polysaccharide may have a molecular weight of about 10,000 and preferably in the range of 1000 to 200,000. A suitable polysaccharide is hydroxypropyl methyl cellulose, which is a cellulose which has been modified to make it more water soluble. Such polysaccharides are non-allergenic and do not discolour fabrics.

The composition may also contain a wetting agent so as to further reduce the droplet size of the spray and to improve surface coverage. Suitable surfactants include alkylpolyglucoside or polyethoxylated sorbitan esters. These compounds have a low potential irritancy to the asthma sufferers.

The composition may also comprise a preservative, such as Bronidox L(trade mark) which is a 10% solution of 5-bromo-5-nitro-1,3-dioxan in 1,2-propylene glycol. This preservative has microbiocidal properties and acts to effectively inhibit the growth of fungi and prevent mould growth.

An advantageous composition according to the invention comprises, 0.500 %w/w of a polysaccharide such as Courgel AG1187(trade mark of Courtaulds Fine Chemicals), 0.250 %w/w of a polyethoxylated sorbitan ester such as Crillet 1(trade mark), 0.275 %w/w of a preservative such as Bronidox L and 98.975 %w/w water.

The invention also provides the use of the above defined compositions for controlling dust mite allergens.

The invention also provides a method of controlling dust mite allergens by applying a composition comprising a coating material able to bind small particles so as to immobilise allergen particles onto the surface. The composition is coated onto the surface by any convenient method such as pad mangling or by spraying of an aqueous solution.

The invention provides the use of the above defined compositions in a method for controlling dust mite allergens.

The composition and method typically produce, in a dust sampling test as described below, a reduction in allergen levels of at least 50 %.

Polysaccharides are defined as Polyoses: a group of complex carbohydrates such as starch, cellulose etc. They may be regarded as derived from x monose molecules by the elimination of x molecules of water. Polysaccharides can be hydrolysed step by step, ultimately yielding a monose.

The invention will now be described by way of example with reference to comparative tests.

It was considered such that a range of naturally-derived materials may be able to immobilise allergens on bedding and upholstery. These could be

applied in a variety of ways however a typical application method could be the application of an aqueous solution with the use of a common trigger type of sprayer, suitable for amateur domestic application.

Although, in principle, a wide variety of polysaccharides could be screened for their ability to immobilise allergens, a range of commercially available polysaccharide from the Courtaulds Fine Chemicals range of water soluble polymers was selected. In particular. a very low molecular weight water soluble cellulose polymer (trade mark Courgel AG 1187) was selected for screening as it was known to have good film forming properties. Its ability to bind small particles preventing subsequent "dusting" also rendered the material suitable for consideration. Various strengths and application rates of product were assembled for trial and although the material may well perform in its own right, it was considered helpful to attempt to improve the characteristics of the formulation further by incorporating a wetting agent. Such an additive would further reduce the droplet size of the spray thus enhancing surface coverage of both the allergens and their substrate. The selection of a suitable surfactant of low potential irritancy to the asthma sufferer was resolved by the inclusion of either recently developed

alkylpolyglucoside surfactant or polyethoxylated sorbitan esters. A preservative was also included within the test formulations to prevent mould growth.

A dust sampling test was undertaken to evaluate the possibility that the polysaccharide formulations would immobilise the release of the house dust mite derived allergen known as Der p 1. Synthetic loop pile carpet pieces, which were not pre-treated with any pesticide, were placed in 8.5cm disposable petri dishes. The surface area of each dish was calculated as 56.75 sq. cm. The rims of the dishes were lined with 2cm paper autoclave tapes leaving a tacky margin to prevent mites escaping from the dishes.

Each dish was seeded with 100 adult mites from a colony of Dermatophagoides pteronyssinus. The infestation rate used in the study was 17621 mites per sq. metre which is within the high mite infestation range (compared with a maximum recorded mite infestation of 200,000 per sq.metre). Tannic acid reference solution from a standard retail product was used as a positive control at the recommended application rate of 25ml per sq. metre.

Two test formulations of the preferred polysaccharide solution were used for the test at 0.1%w/w and 0.5%w/w respectively and sprayed at the same dosage rate as the tannic acid control.

Dust sampling was performed by vacuuming the carpet dishes covering the whole area once. The collecting device which had a 50 micron wire mesh bottom was mounted to the tip of a Panasonic Electronic MC-E97 vacuum cleaner hose. The diameter of the collecting tube was 21mm and had a detachable cap at the base. A whatman GF/A glass microfibre filter, with a diameter of 25mm and pore size of 1.5 microns was attached above the wire mesh of the collecting device to trap dust and allergen particles. Dishes containing carpet pieces were incubated at 75%Rh for 48 hours together with 0. 1g of laboratory mite food to make the habitat suitable for mite survival. 10 adult mites were seeded to each dish and the dishes were further incubated at 75%Rh and room temperature for 14 days. An adult mite has been recorded to excrete about 20 pellets per day which contain approximately 100pg of Der pl in each pellet. 100 mites incubated for 14 days under optimum conditions were calculated to excrete approximately 2.8 micrograms of Der pI in each dish at the rate of 499 micrograms per sq. metre.

After the incubation each dish was weighed and then sprayed with either tannic acid solution or one of the test solutions. The experiment included a set of control dishes which were not treated with any of the solutions tested. Five replicates were made for each observation

and the dishes left to dry at room temperature overnight.

Each dish was vacuumed over the whole surface once and the dust collected and weighed and transferred to specimen bottles and placed at - 18 °C for one week to kill off any surviving mites. After thawing the dust, each sample was eluted with 2ml of 0. 125M ammonium hydrogen carbonate buffer to extract the allergens. The solution was mixed and left for 6 hours and then centrifuged at 6000rpm for 5 minutes. The supernatant fluid was pipetted out and stored at -18 °C until Der pl allergen analysis was performed using the ELISA system.

Very high levels of the allergen were recorded from each of the untreated control dishes. The mean Der pl level for the controls was 4669 micrograms per sq.m. which is approximately 10 times higher than expected. This may have been due to the increase in mite population in each dish during the 14 day incubation period.

Dishes that were treated with tannic acid solution had reductions in the allergen level of 23.7% by comparison with untreated controls on average. This result was similar to results previously observed in field trials by other workers (Ref 1.) and were therefore considered valid. Variations in individual results were attributed to the possibility that the formulation may not have adequately penetrated the substrate or the

variation in allergen or mite levels before treatment.

Replicates treated with 0.1 %w/w polysaccharide exhibited reductions in allergen levels of the order of 16.8%. When compared with 23.7% for the tannic acid solution this indicates a beneficial effect even at such a low dosage.

The dishes treated with 0.5% polysaccharide exhibited Der p1 level reductions in all replicates which were much lower than the controls. The mean allergen level was reduced to 2006 micrograms per sq. m. which was a 57% reduction and was, therefore, about twice as effective as tannic acid in lowering the allergen levels in the treated carpet samples.

A dose titration study of the 0.5% polysaccharide allergen control spray (PACS) has been performed in order to more closely determine the dosage required to control house dust mite allergens in the home.

PACS was tested for its efficacy in reducing house dust mite allergens in carpet pieces under laboratory conditions. In samples treated with 0.5% PACS there was a 35.7 - 65.6% reduction of Der.p1 in the 5 replicates. The dosage used ranged from 0.22 to 0.29g/56cm^2. Higher dosages of 0.26 and 0.29g seemed to produce a higher reduction in the allergen levels.

Synthetic loop pile carpet pieces without any pre-treatment with pesticides were placed in 8.5cm Petri dishes and the rim was lined with tape to prevent mites from escaping. They were conditioned at 75%Rh together with 0.1g of mite food to promote mite growth. Each dish with a surface area of 56.75cm^2 was seeded with 50 adult mites from the Cambridge Medical Entomology Centre colony of Dermatophagoides pteronyssinus. The infestation rate used in the study of >800/m^2 was a high level encountered in the U.K.

The mites were incubated at 75%Rh and room temperature for 2 weeks and allowed to deposit their faecal allergen on to the substrate. The dishes were then frozen overnight to kill off mites and the carpet samples were used for testing with PACS.

Each carpet sample was sprayed with either 1, 2, 3, 4 or 5 trigger doses of 0.5% PACS and the amount deposited was weighed. They were then air-dried for 1 hour and the dust from each sample was vacuumed for about 30 seconds covering the whole area. Dust was collected in a tube containing a glass microfibre filter disc of 1μm pore size graded-density, Whatman GMF150 supported on a 50μm wire mesh. Three replicates were performed for each dosage and they were accompanied by 3 untreated dishes to determine the base line of Der.pl for

calculation of percentage reduction.

The filter dishes were retrieved and mite allergen were eluted by mixing each disc with 2ml of 0.125M ammonium carbonate buffer for 2 hours. The mixture was centrifuged at 7000 rpm for 5 minutes and the supernatant fluid was pipetted out and stored at -20°C until the Der.pl was performed. Quantitative analysis of the allergen level of each sample was determined by using the ELISA system. A scatter diagram was drawn with dose and allergen levels to determine whether there was any correlation between the two variables and the analysis of variance between dosages was performed using the Mann-Thitney 'U' test. Der.pl levels from the ng/ml were converted into $\mu g/m^2$ by multiplying the value with a factor of 35.2 obtained by calculation from 2ml amount of dust extract obtained from a $56.75cm^2$ carpet sample and the dilution of 1:100 used for the assay. The percentage reduction from the untreated samples was then calculated.

The dosage of PACS and Der.pl levels obtained from the dust samples were tabulated. The dust samples collected from the untreated carpets contained 22-200ng/ml of Der.pl. This variation in the base line allergen levels may have been due to the differences in mite population at the end of the 14 day incubation

period. Although adult mites were used, there could still have been differences in the sex ratio and gravidity which would result in higher numbers in samples that contained higher gravid females.

A single application with PACS obtained a result of 27-100ng/ml of the allergen and did not have a significant reduction from the base line levels whereas two or more applications had substantial reductions. The wide range of Der.pl was still present with 2 and 3 applications (15.5 - 74 and 6.6 - 60 ng/ml respectively) but it disappeared at much higher dosages. This reflects the need for more PACS to bind higher levels of mite allergens which might exist in some of the replicates as was observed with the untreated controls.

There was a more uniform reduction in Der.pl levels 9-14 ng/ml obtained with 4 applications of PACS and 5-14.5 ng/ml with 5 applications, despite the assumption that there were varying levels of mite allergens in the carpet samples before the treatment.

The scatter diagram for dose-allergen level showed a negative correlation with the coefficient (r) of -0.76 and a marked decrease in the Der.pl levels was noted with higher dosages. There was a significant difference between dose 1 and dose 5 using the Mann-Whitney test at 0.05 level as well as between doses 2 and 5, but not

between doses 3 and 5.

The PACS treatment to infested carpet samples lowered the mite-allergen levels in the dust obtained by vacuuming. There was a does related fall in Der.pl levels especially noted with higher doses. It appears that 3 applications of PACS with the dosage of 0.73g, 0.83g and 1.07g to 56.75cm^2 carpet samples was sufficient to bind the allergens. However, in one of the replicates there was a 60ng/ml of Der.pl equivalent to 2112 $\mu g/m^2$ which was quite high. This might be due to higher pre-existing levels of mite allergen which was not sufficiently bound with the dose applied. In one of the untreated samples there was 7040$\mu g/m^2$ of mite allergens which was exceedingly high. For such high levels, it would be necessary to apply at least 4 times since the allergen levels obtained by applying 4 and 5 times were uniformly reduced and there was no statistical difference between these two dosages.

CLAIMS

1. A composition for controlling dust mite allergens, comprising a coating material able to bind small particles and which can be applied to a surface so as to substantially immobilise allergen particles on the surface.

2. A composition for controlling dust mite allergens as claimed in claim 1, wherein the coating material is water soluble.

3. A composition for controlling dust mite allergens as claimed in claim 1 or 2, wherein the coating material is a polysaccharide.

4. A composition for controlling dust mite allergens as claimed in claim 3, wherein the polysaccharide has a molecular weight in the range of 1000 to 200,000

5. A composition for controlling dust mite allergens as claimed in claim 4, wherein the polysaccharide has a molecular weight of about 10,000.

6. A composition for controlling dust mite allergens as claimed in claim 4, 4 or 5, wherein the polysaccharide is hydroxypropyl methyl cellulose.

7. A composition for controlling dust mite allergens as claimed in claim 1 or 2, wherein the coating material includes a polyvinylalcohol (PVA).

8. A composition as claimed in any previous claim wherein the composition comprises a wetting agent.

9. A composition as claimed in claim 8, including at least one surfactant selected from the group consisting of alkylpolyglucoside or polyethoxylated sorbitan esters.

10. A composition as claimed in any previous claim, including at least one preservative.

11. A composition as claimed in claim 10, wherein the preservative includes Bronidox L(trade mark).

12. A composition according to claim 1 comprising 0.500 %w/w of a polysaccharide, 0.250 %w/w of a polyethoxylated sorbitan ester, 0.275 %w/w of Bronidox L and 98.975 %w/w water.

13. Use of a composition as claimed in any previous claim in a method of controlling dust mite allergens.

14. A method of controlling dust mite allergens comprising applying a composition comprising a coating material able to bind small particles to a surface so as to substantially immobilise allergen particles on the surface.

15. A method of controlling dust mite allergens as claimed in claim 14, wherein the composition is applied by pad mangling or by spraying of an aqueous solution.

16. A method of controlling dust mite allergens as claimed in claim 14 or 15 and comprising applying a composition as claimed in any one of claims 1 to 12

The Patent Office

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Claims searched: 1-16

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Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): A5E (EN)

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Other: Online: WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	WO 93/18404 A1 (UNIV. COLL. DUBLIN), see eg. claims 1 and 3	1,13 and 14 at least
X	EP 0619323 A1 (SCHERING-PLOUGH), see eg. claims 1 and 3	1,13 and 14 at least

X Document indicating lack of novelty or inventive step
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P Document published on or after the declared priority date but before the filing date of this invention.
E Patent document published on or after, but with priority date earlier than, the filing date of this application.